Phytochemistry, Vol. 26, No. 11, pp. 2901–2903, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

A SIMPLE HISTOCHEMICAL METHOD FOR LOCATING ENZYMES IN PLANT TISSUE USING NITROCELLULOSE BLOTTING

JANICE SPRUCE, ALFRED M. MAYER* and DAPHNE J. OSBORNE†

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA, U.K.

(*Received* 30 *March* 1987)

Key Word Index—Nitrocellulose blotting; histochemistry; plant enzymes; abscission zones.

Abstract—The blotting of fresh tissue sections onto nitrocellulose and the detection of enzyme activities on the blots for polyphenol oxidase, peroxidase, glycosidases, dehydrogenase and phosphatase activity has been shown successfully using the method. The applicability to various tissues and enzyme localization is discussed.

INTRODUCTION

The histochemical and phytochemical localisation of plant enzymes is well developed at the microscopic level [1] but can nevertheless often be time consuming and tedious. The plant tissues frequently contain the native substrates of the enzymes to be detected or compounds that specifically inhibit the enzymes. In addition many plant tissues contain substances, such as phenolics, which bind to the proteins and generally inhibit enzyme activity. The speedy localization of enzyme activities in tissue sections is often desirable as well as a relative measure of the activity between different cell types as, for example, in abscission zones [2].

A simple rapid method to achieve this without first processing the tissue, and without interference by inhibitors or substrates would therefore be very valuable. We describe such a method utilising the properties of nitrocellulose to absorb and retain proteins, as used in various molecular biological blotting techniques.

RESULTS AND DISCUSSION

As can be seen from Fig. 1 sections through the petiole of the primary leaf of the bean (*Phaseolus vulgaris*) in the region of the distal abscission layer or of the lower part of oil palm fruit (*Elaeis guineensis*), also in the region of the abscission zone, clearly show that it is possible to detect enzyme activities blotted on to nitrocellulose. The blotting was achieved by simply pressing a cut section lightly on to the membrane and then developing with specific histochemical reagents. The data in Fig. 1 show representative examples of the assay for some of nine enzymes and in the two different tissues.

We have assayed for α- and β-glycosidase, α- and β-galactosidase, acid phosphatase, polyphenol oxidase, peroxidase, and glutamate and malate dehydrogenase. The localisation of some of the enzymes and the wide spread of others, can be seen.

For example, polyphenol oxidase (Fig. 1a) and phosphatase (not shown) are present throughout the oil palm section while β-glucosidase is detected only in the abscission zone (Fig. 1b). Although some peroxidase is present throughout the section, a clear zone of high activity was noted in the abscission zone (Fig. 1c). In the bean petiole polyphenol oxidase (not shown) and glutamete dehydrogenase activity were observed particularly in the region of the pulvinus (Fig. 1f) while peroxidase (Fig. 1d) is widely distributed. Differences in activity could be clearly seen on either side of the abscission layer.

The method should be applicable for any enzyme which can be transferred to nitrocellulose and for which a histochemical assay exists, using a substrate that results in the formation of insoluble or partially soluble coloured or fluorescent product.

Microscopical examination of the tissue blots indicates that proteins located in individual cells are giving enzyme reactions. Indeed different cell types such as vascular tissue can be readily distinguished on the blots; the restriction of β-glucosidase to the abscission layer of the oil palm is an example.

The method should find wide application for the localisation of enzymes in specific differentiating or differentiated plant tissues and could be particularly useful in phytopathological studies where it is desired to locate host or parasite enzyme in tissues.

EXPERIMENTAL

2 cm squares of nitrocellulose membrane filters B A 28, (Schleicher & Schüll, 0.45 μm pore size) were soaked in distilled H_2O, placed on microscope slides and blotted dry with a tissue. A 2–3 mm section through the plant tissue was placed on the membrane and then lightly pressed on it using an additional microscope slide. The section was removed carefully and the membrane thoroughly rinsed with distilled H_2O to remove non-proteinaceous material or soluble compounds and lightly pressed with a tissue to remove excess water. About 0.2 ml of the appropriate substrate or reagent was next spread over the surface of the membrane. The slides were incubated in a moist atmosphere for the reaction to proceed, the substrate was then rinsed off, and if necessary a developing reagent added. For fast reactions with immediate colour production the substrate was

*Permenent address: Department of Botany, Hebrew University, Jerusalem 91904, Israel.

†Author to whom correspondence should be addressed.

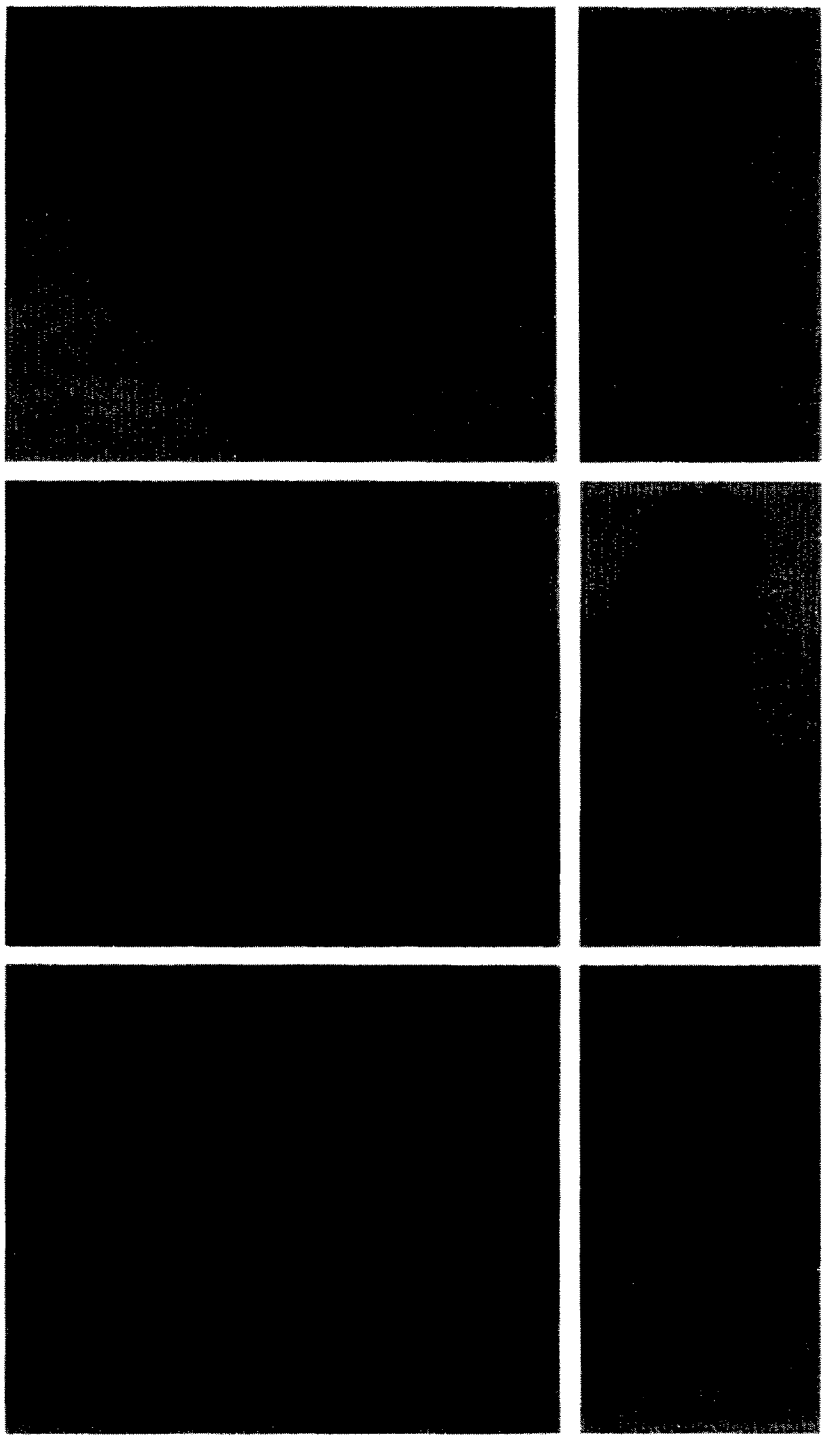

Fig. 1. Photographs of histochemical blots of oil palm fruit and bean leaf petioles. (a–c) Sections through oil palm fruit across fruit across abscission zone. (d–f) Sections through petiole, abscission zone and pulvinus of bean. (d, e, partially separated zones). (a) Polyphenol oxidase; (b) β-glucosidase; (c, d) peroxidase; (e) malate dehydrogenase; (f) glutamate dehydrogenase. Darkened or black areas show sites of enzyme activity. 1 = Abscission zone; 2 = proximal side; 3 = hollow pith (bean petiole).

added and rinsed off as soon as colour development was optimal, e.g. polyphenoloxidase or peroxidase. Alternatively the nitrocellulose blot can be placed (blot side upwards) on to a piece of filter paper moistened with substrate. This gives very good resolution and localization of enzyme activity.

For work with oil palm fruit the substrates were made up in pH 4.8 acetate buffer 20 mM, and for the bean leaf petioles pH 6.1 Pi buffer, 100 mM. We have used the following reagents from Sigma, London for detecting enzyme activity: for polyphenol oxidase the reagent was 3,4-dihydroxyphenylalanine 20 mM [3], for per-

oxidase we used 0.2% guaiacol and 0.2% H_2O_2, but 3,3'-diaminobenzidine is equally effective. For α- and β- glucosidase and galactosidase the substrates were the corresponding bromonaphthol glycosides as described in refs [4, 5] using 3 mg/10 ml. For acid phosphatase we used 1 mg/2 ml naphthol-AS-BI-phosphate. In these tests the substrate was washed off after incubation for some 3–4 hr at 26° and the visualizing reagent Fast Blue B 1 mg/ml added and left on the membrane for 4 min. The dehydrogenases were detected with tetrazolium nitroblue 0.03% in the presence of 0.002% PMS, 1.0 mM NAD and 150 mM glutamate or 100 mM malate [6]. All membranes were finally rinsed, excess H_2O removed with a tissue, and air-dried. Placed between two slides membranes could be kept in the dark for several weeks without deterioration.

The dried membranes are readily photographed, using appropriate light filters.

Note added in proof: With C. C. McCready we have now successfully extended this tissue blot method for cell recognition by immunogold blotting procedures using Janssen Auro Probe BL Plus followed by silver enhancement.

Acknowledgement—We wish to acknowledge financial support from Unilever Plantations Group.

REFERENCES

1. Gahan, P. B. (1984) *Plant Histochemistry and Cytochemistry.* Academic Press, New York.
2. Osborne, D. J., and McManus, M. T. (1986) *Curr. Topics Develop. Biol.* **21**, 383.
3. Mayer, A. M. and Harel, E. (1979) *Phytochemistry* **18**, 193.
4. Rutenburg, A. N., Goldbang, J. A., Rutenburg, S. H. and Lang, R. T. (1960) *J. Histochem. Cytochem.* **8**, 268.
5. Seligman, A. M., Tsou, K-C., Rutenburg, S. H. and Cohen, R. B. (1954) *J. Histochem. Cytochem.* **2**, 209.
6. Shaw, C. R. and Prasad, R. (1970) *Biochem. Genet.* **4**, 297.